

PATENT
674521-2001.1AMENDMENT

Please amend the application without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE CLAIMS

1. (Currently amended) [[An]] The isolated and purified retrotransposon according to claim 12 comprising the gag and pol ORF in the same phase and found as an extrachromosomal DNA molecule having a copy number of 40-150 free DNA copies per cell.
2. (Currently amended) [[A]] The retrotransposon according to claim [[1]] 12 which is linear.
3. (Currently amended) [[A]] The retrotransposon according to claim 2 which is double stranded.
4. (Currently amended) [[A]] The retrotransposon according to claim [[1]] 12 which is isolated from fungi or yeast.
5. (Currently amended) [[A]] The retrotransposon according to claim 12, comprising the genetic material encoding at least one polypeptide positioned between at least two long terminal repeats, wherein the retrotransposon is present at a copy number of between 40-150 free DNA copies of itself per cell, and wherein the free DNA copies are capable of integrating into the DNA of a host genome.
6. (Currently amended) [[A]] The retrotransposon according to claim 5 which is isolated from fungi or yeast.
7. (Withdrawn) A method of introducing DNA into the genome of a cell which method comprises introducing a transposable element comprising a nucleotide sequence encoding a desired protein located between two long terminal repeats sequences having the sequences illustrated in Figure 2B, which element is such that it can insert into the genome of said cell in the presence of an integration factor.
8. (Withdrawn) A method according to claim 7 wherein said integration factor comprises an integrase which optionally is itself included in said transposable element and which integrase is derived from the POL region of said pCAL retrotransposon.
9. (Previously amended) A transposable element for introducing a desired DNA sequence into the genome of a cell, having the sequence identified in SEQ ID NO: 3 comprising an internal domain for receiving a nucleotide sequence encoding a desired protein, said internal

PATENT
674521-2001.1

domain comprising the gag and pol ORF in the same phase and flanked by two terminal repeat regions.

10. (Currently amended) A DNA transfer construct comprising:
 - a) a transposable element for introducing a desired DNA sequence into the genome of a cell, having the sequence identified in SEQ ID NO: 3 comprising an internal domain for receiving a nucleotide sequence encoding a desired protein, said internal domain comprising the gag and pol ORF in the same phase and flanked by two terminal repeat regions, said transposable element being capable of integrating into the genome of a cell in the presence of an integration factor; and
 - b) a nucleotide sequence encoding an integration factor.
11. (Currently amended) [[A]] The transposable element according to claim 9 comprising an open reading frame encoding an integration factor which is an integrase protein and which is optionally encoded by a nucleotide sequence within the POL region of the retrotransposon of Figure 2B.
12. (Currently amended) An isolated and purified retrotransposon comprising a nucleotide sequence selected from the group consisting of:
 - (a) SEQ ID NO: 3;
 - (b) [[A]] a nucleotide sequence with at least 65% similarity with the LTR and POL region of SEQ ID NO: 3; and
 - (c) [[A]] a nucleotide sequence that hybridizes under stringent conditions to SEQ ID NO: 3.
13. (Original) The integrated form of the retrotransposon claimed in claim 12 comprising the integrated form being herein designated TCa2.
14. (Original) An expression vector comprising the retrotransposon of claim 1, 5 or 12.
15. (Withdrawn) A method of gene disruption or altered expression comprising integrating a retrotransposon of any one of claims 1, 5 or 12 into a site or sites in a yeast or fungus or *Candida* wherein the retrotransposon contains elements that cause gene disruption or altered expression at the site or sites; and, optionally the gene disruption or altered expression is non-reversible.

PATENT
674521-2001.1

16. (Withdrawn) A gene discovery method comprising integrating a retrotransposon of any one of claims 1, 5 or 12 into a site or sites in a yeast or fungus or *Candida* wherein the retrotransposon contains elements that cause gene disruption or altered expression at the site or sites, and, optionally the gene disruption or altered expression is non-revertible; and, mapping the gene or genes disrupted or whose expression has been altered, by the retrotransposon.

17. (Currently amended) A carrier construct comprising the retrotransposon of claim 1, 4, 5, 6 or 12.

18. (Currently amended) A transformation and expression construct for fungi or yeast or *Candida* comprising a retrotransposon of claim 1, 4, 5, 6 or 12.

19. (Previously amended) A nucleic acid fragment selected from the group consisting of:

- (a) a nucleic acid sequence positioned between at least two terminal repeats of the sequence of pCal as described in GenBank accession number AF007776;
- (b) a nucleic acid sequence with at least 65% similarity with the LTR and POL region of the sequence of (a); and
- (c) a nucleic acid sequence that hybridizes under stringent conditions to the nucleotide sequence of (a).

20. (Currently amended) [[A]] The nucleic acid fragment according to claim 19 in which the nucleic acid sequence comprises a functional POL gene.

21. (Currently amended) [[A]] The nucleic acid fragment according to claim 19 in which the nucleic acid sequence comprises two long terminal direct repeats flanking a series of genes in the order gag (group antigen), pol (polyprotein) where the pol sequence comprises an aspartic protease, an integrase and a reverse transcriptase/RNaseH.

22. (Withdrawn) A functional optionally temperature sensitive inducible promoter isolated from a retrotransposon of claim 1, 5 or 12.

23. (Withdrawn) A retrotransposon selected from the group consisting of retrotransposons 1-28, whose sequences are given in accompanying figures 17-48, and 71.

24. (Withdrawn) A method of assigning a function to a nucleotide sequence which method comprise providing said sequence between the long terminal repeat sequences of the transposable element according to claim 1, 5 or 12 and introducing it into said cell and

PATENT
674521-2001.1

monitoring for the presence of an altered phenotype of said cell compared to a cell which has not had said nucleotide sequence introduced therein.

25. (Withdrawn) A method for gene disruption or altered expression comprising disrupting a gene by active retrotransposition into a new site or sites in the *Candida* genome of a retrotransposon, wherein the gene disruption or altered expression is optionally non-revertible.

26. (Withdrawn) A method for discovering a gene comprising disrupting a gene by active retrotransposition into new site or sites in the *Candida* genome of a retrotransposon, wherein the gene disruption is optionally non-revertible; and, mapping the gene disrupted.

27. (Withdrawn) An immunological, or immunogenic, or vaccine or therapeutic composition comprising a carrier or diluent and the expression vector of claim 14 wherein the vector expresses an antigen, or an epitope of interest or a therapeutic.

28. (Withdrawn) The composition of claim 27 comprising an immunological, immunogenic or vaccine composition, wherein the vector expresses an antigen or an epitope of interest.

29. (Withdrawn) The composition of claim 27 comprising a therapeutic composition, wherein the vector expresses a therapeutic.

30. (Withdrawn) A method for inducing an immunological response in a host including an animal or a human comprising administering to the host the composition of claim 27.

31. (Withdrawn) A method for inducing a therapeutic response in a host including an animal or human comprising administering to the host the composition of claim 28.

32. (Withdrawn) A method for detecting the presence of *Candida* comprising detecting the presence in a sample of a retrotransposon as claimed in any one of claims 1, 5 or 12.

33. (Cancelled)

34. (Cancelled)

35. (Currently amended) The retrotransposon of claims 1, 5 or 12 ~~[[34]]~~, wherein the retrotransposon comprises a nucleotide sequence having four tandem repeats of the sequence GAAAAA.

36. (Previously added) The transposable element of claim 9, wherein the transposable element comprises a nucleotide sequence having four tandem repeats of the sequence GAAAAA.

PATENT
674521-2001.1

37. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 70% similarity with the LTR and POL region of SEQ ID NO:3.
38. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 75% similarity with the LTR and POL region of SEQ ID NO:3.
39. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 80% similarity with the LTR and POL region of SEQ ID NO:3.
40. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 85% similarity with the LTR and POL region of SEQ ID NO:3.
41. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 90% similarity with the LTR and POL region of SEQ ID NO:3.
42. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 95% similarity with the LTR and POL region of SEQ ID NO:3.
43. (New) The retrotransposon of claim 12, wherein the nucleotide sequence of (b) has at least 97% similarity with the LTR and POL region of SEQ ID NO:3.
44. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 70% similarity with the LTR and POL region of the sequence of (a).
45. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 75% similarity with the LTR and POL region of the sequence of (a).
46. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 80% similarity with the LTR and POL region of the sequence of (a).
47. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 85% similarity with the LTR and POL region of the sequence of (a).
48. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 90% similarity with the LTR and POL region of the sequence of (a).
49. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 95% similarity with the LTR and POL region of the sequence of (a).
50. (New) The nucleic acid fragment of claim 19, wherein the nucleic acid sequence of (b) has at least 97% similarity with the LTR and POL region of the sequence of (a).